



Polysaccharide isolated from Aloe vera gel suppresses ovalbumin-induced food allergy through inhibition of Th2 immunity in mice

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ABSTRACT

An allergic reaction occurs when the immune system overreacts to harmless substance called allergen that gains access to the body. Food allergy is a hypersensitive immune reaction to food proteins and the number of patients with food allergy has recently increased. *Aloe Vera* is used for wellness and medicinal purposes. In particular, *Aloe vera* has been reported to enhance immunity. However, the effect of *Aloe vera* on food allergy is not yet known. In this study, we investigated the effects of processed *Aloe vera* gel (PAG) containing low molecular weight Aloe polysaccharide (AP) on ovalbumin (OVA)-induced food allergy in mice. Allergic symptoms, rectal temperature, and diarrhea were measured in OVA-induced food allergy mice. Other allergic parameters were also analyzed by RT-PCR, ELISA, flow cytometry, and other biochemical methods. As the results, PAG suppressed the decrease of body temperature, diarrhea, and allergic symptoms in OVA-induced food allergy mice. PAG also reduced serum concentrations of type 2 helper T cell (Th2) cytokines (Interleukin-(IL)-4, IL-5, and IL-13) as well as histamine, mast cell protease-1 (MCP-1), and immunoglobulin (Ig)E. PAG blocked the degranulation of mast cells and infiltration of eosinophils in intestine. Furthermore, PAG suppressed the population of Th2 cells in spleen and mesenteric lymph nodes. PAG also increased the production of IL-10 and population of type 1 regulatory T (Tr1) cells in mice with food allergy. Taken together, our findings suggest that PAG suppressed Th2 immune responses through, at least partially, stimulating the secretion of IL-10 in food allergy mice.

1. Introduction

Allergic diseases such as food allergy, allergic rhinitis, atopic dermatitis, and allergic asthma have been increasing in recent years. Symptoms of allergic disease include urticaria, angioedema, dermatitis, diarrhea, vomiting, abdominal pain, nasal congestion, and sneezing. Among allergic diseases, food allergy is an allergic disease caused by hypersensitive immune response to food-derived antigen proteins. Food allergy not only reduce the quality of life, but also can cause lethal symptoms such as anaphylactic shock. The prevalence of food allergy in the United States of America is reported to be 6–15% in children and 4% in adults. The major causes of food allergy are reported to vary by age. In children, eggs, milk, peanuts, fish, and nuts are the main causes.

In adults, peanuts, nuts, fish, and crustaceans are the causative agents in 85–90% of patients [1].

The prevalence of food allergy worldwide is increasing, and the need for further research on the treatment of this disease is being emphasized. In clinical practice, strict dietary restriction of causative foods is the main cure. Therefore, recently, the social demand for the development of new therapy is increasing. The most ideal treatment of allergic diseases is to induce immune tolerance to allergy-causing substances called allergen in the body. Studies on the induction of immune tolerance and regulation of allergy response to allergens have been actively conducted using food allergy animal models [2–4]. In particular, recently, a therapeutic approach through controlling immune function in atopic dermatitis and food allergy has been attempted

Abbreviations: PAG, processed *Aloe vera* gel; AP, Aloe polysaccharide; OVA, ovalbumin; Ig, immunoglobulin; MCP-1, mast cell protease-1; Th1, type 1 helper T cells; Th2, type 2 helper T cells; PBS, phosphate buffered saline; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; IFN- γ , interferon- γ ; Tr1, IL-10-producing type 1 regulatory T cells

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worldwide, but it still shows limitations as a therapeutic application. At this point, it is necessary to hasten the discovery of medicine based on the regulation of immune response for the treatment of food allergy.

In recent years, *Aloe vera* has been used as a therapeutic and health functional food in various diseases. It is also known to be a natural material for the regulation of immune responses, the activation of intestine function, and skin moisturizing effect [5–9]. In particular, the *Aloe vera* polysaccharides are known as substances that help modulate immunity in human body. However, the study on the effect of *Aloe vera* polysaccharide in food allergic diseases remains to be discovered. In this study, we investigated the effect of *Aloe vera* polysaccharide on food allergy and its mechanism in mice.

2. Materials & methods

2.1. Preparation of processed *Aloe vera* gel

The processed *Aloe vera* gel (PAG) was prepared as described previously [10,11]. Briefly, *Aloe vera* gel is reacted with cellulase and then passed through charcoal column to remove anthraquinones and other colored substances. Finally, it was ultrafiltrated using Amicon Stirred Cell (MWCO, 5000 Da, Merck, Kenilworth, NJ). The yield of PAG from *Aloe vera* gel is approximately 0.5%. In order to eliminate the possibility of endotoxin contamination, we removed endotoxin by the method reported previously [12]. It was confirmed that the PAG contains less than 0.1 ng endotoxin/mg PAG. In addition, we used a TSKgel G4000 PWxl column (7.8 mm × 30 cm; Tosoh Bioscience, Shiba, Japan) to determine the molecular weight distribution of PAG. As described by Im et al. [11], the molecular weight distribution of PAG polysaccharides was found to be much smaller than that of *Aloe vera* gel. The molecular weight of most polysaccharides contained in *Aloe vera* gel was greater than 1 MDa, while that of most polysaccharides in PAG was less than 500 kDa. To be precise, the molecular weight distribution of PAG consisted of more than 75% of 10–500 kDa polysaccharides. The PAG was dissolved in 1 × phosphate buffered saline (PBS) for following experiments.

2.2. Mice

BALB/c mice (female, 5 weeks old) were obtained from Orient (Seongnam-si, Korea) and housed in a specific pathogen-free animal facility at Konkuk University (Seoul, Korea) with sterilized diet and autoclaved water ad libitum. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Konkuk University.

2.3. Induction of food allergy mice

Food allergy mouse was made by ovalbumin (OVA) as described in Fig. 1A. Mice were injected i.p. twice with 10 µg of OVA plus 1 mg of Alum (Sigma-Aldrich, St. Louis, MO) on days 0 and 7. One week later, 10 mg OVA dissolved in 200 µl PBS was administered via oral gavage on days 14, 16, 18, and 21, respectively. PAG was orally administered around 9 a.m. daily until the day before the challenge through the entire experimental procedure. The administration of OVA for the sensitization was administered around 2 p.m. each time. One week after the last sensitization, mice were challenged with 50 mg OVA in 200 µl PBS via oral gavage after overnight fasting. The change of rectal temperature was assessed for 1 h after challenge using a digital thermometer TESTO 925 (Testo AG, Germany). Symptom score was estimated as follows: 0, no symptoms; 1, scratching around nose and head; 2, puffiness around eyes and mouth; 3, wheezing, labored respiration, cyanosis around mouth and tail; 4, no activity after prodding or tremor and convulsion; 5, death. In addition, diarrhea occurred within approximately 30 min after the OVA challenge in the mice. We measured diarrhea when measuring body temperature through the anus.

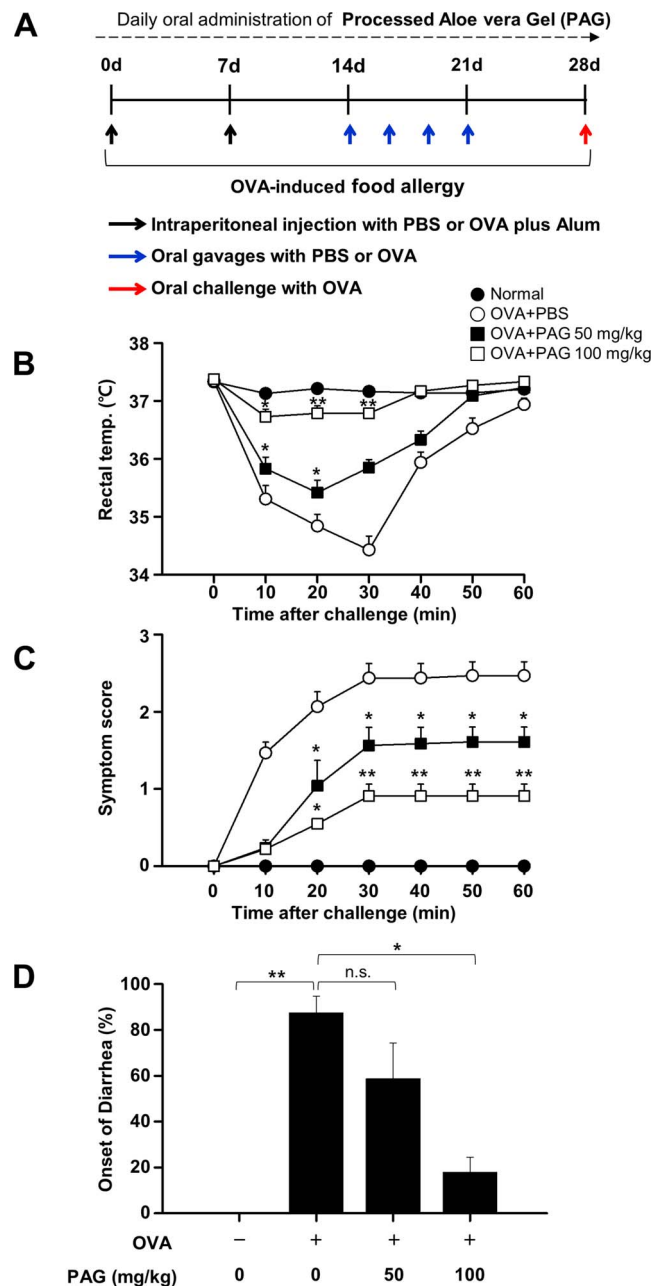


Fig. 1. PAG suppresses OVA-induced food allergic responses. (A) Protocol for the induction of OVA-mediated food allergy. Female mice (5 weeks old) were sensitized and challenged with OVA as described in “Materials and Methods”. (B–D) Analysis of rectal temperature (B), symptom score (C), and onset of diarrhea (D) were assessed at the indicated times. Data were presented as the mean ± s.e.m. obtained from 3 individual experiments. n = 5 mice per group. *p < 0.05; **p < 0.01; n.s., not significant. OVA, Ovalbumin; PAG, Processed *Aloe vera* gel.

2.4. Flow cytometry analysis

Cells were isolated from spleen, mesenteric lymph node, and jejunum. For detection of intracellular interleukin (IL)-4 and interferon (IFN)-γ, isolated cells were cultured with 1 µg/ml anti-CD3/CD28 with or without 200 µg/ml OVA for 72 h or indicated times and then phorbol 12-myristate 13-acetate (PMA, 50 ng/ml, Sigma-Aldrich), ionomycin (500 ng/ml, Sigma-Aldrich), and Brefeldin A (3 µg/ml, eBioscience, San Diego, CA) were treated for 4 h. Next, the cells were fixed and permeabilized using a fixation/permeabilization concentrate kit (eBioscience, Waltham, MA) and then incubated with antibody against IL-4, IFN-γ, or IL-10 at 4 °C for 30 min. The antibodies against CD3

(17A2), CD4 (GK1.5), and CD11b (M1/70) were purchased from eBioscience. Anti-Siglec-F (552126) was obtained from BD Biosciences (San Jose, CA). Stained cells were analyzed with FACSCalibur (Becton Dickinson, San Jose, CA) and the data were analyzed with FlowJo version 10 software (TreeStar, Ashland, OR).

2.5. Measurement of OVA-specific antibody

Serum was collected 60 min after the last challenge and stored at -80°C before use. OVA-specific immunoglobulin (Ig)E, IgG1 and IgG2a levels were measured by ELISA. A microtiter plate was incubated with 50 μg OVA per well in 0.1 M sodium carbonate (pH 9.5) overnight at 4°C . On next day, the plate was washed twice with PBS and incubated for 1 h with 10% FBS in PBS. Then, serum samples were added to the plate and incubated at room temperature (RT) for 2 h. The plate was incubated with 2 μg biotin-labeled antibodies against IgE, IgG1, or IgG2a (BD Biosciences) for 1 h at RT. Next, the solution was washed three times with 0.5% tween 20 in PBS and the plate was incubated for 30 min at RT after addition of streptavidin-horseradish peroxidase solution (BD Biosciences). After that, the plate was washed five times and TMB substrate solution (100 μl per /well) was added (GenDEPOT, Barker, TX). Absorbance was measured at 450 nm.

2.6. Analysis of histamine and cytokine in serum

Histamine was measured using an ELISA kit (Beckman Coulter, Fullerton, CA). MCP-1 was measured using an ELISA kit (Ready Set Go, eBiosciences) and other cytokines were measured by an ELISA kit (BD Bioscience). Those ELISA analyses were performed according to the manufacturer's protocol.

2.7. Quantitative real-time PCR

Total RNA was extracted from jejunum using RNA isolation kit (ThermoFisher Scientific, Waltham, MA) and cDNA synthesis was performed. All primers for murine IL-4, IFN- γ , and IL-10 were obtained from LightCycler[®]480II System and real-time PCR was performed with LightCycler[®] 480 SYBR green I master mix (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. PCR amplification of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed on each sample for normalization between samples. The intensity of the expression of each gene quantitated using LightCycler[®]480 Software 1.5.0 (Roche Diagnostics, Mannheim, Germany) and all samples were measured in triplicates for statistical analysis. Primers used: IL-4 (Forward: 5'-CCT ATC GAT GAA TCC AGC CAT-3', Reverse: 5'-CAT CGG CAT TTT GAA CGA GGT CA-3'); IFN- γ (Forward: 5'-CCT GCG GCC TAG CTC TGA G-3', Reverse: 5'-GCC ATG AGG AAG AGC TGC A-3'); IL-10 (Forward: 5'-ATT TGA ATT CCC TGG GTG AGA AG-3', Reverse: 5'-CAC AGG GGA GAA CGA TGA CA-3'); GAPDH (Forward: 5'-CAA AGT GGA GAT TGT TGC CA-3', Reverse: 5'-GCC TTG ACT GTG CCG TTG AA-3').

2.8. Histological analysis

The extracted jejunums were fixed with 4% paraformaldehyde and then the tissue was dehydrated by the stepwise increased concentration of ethanol (70–100%). Next, the tissue was washed twice with xylene for 3 min, and then embedded in paraffin. The paraffin block was cut to a thickness of 5 μm and stained with toluidine blue. The number of mast cells in jejunum was measured in 10 sections from five mice. The images were obtained with Olympus DP 70 Microscope Digital Camera (Olympus DP 70, Tokyo, Japan) at $\times 400$ magnification, High-power filter (HPF).

2.9. Effect of PAG on activation-induced apoptosis of CD4⁺ T cells

Splenocytes were isolated from whole spleen of BALB/c. The cells were cultured with 1 $\mu\text{g}/\text{ml}$ anti-CD3/CD28 antibodies for 48 h with or without 100 $\mu\text{g}/\text{ml}$ PAG. Next, CD4⁺ T cells were sorted using a CD4 mAb-microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). The cells were harvested, washed with PBS twice, and then stained with anti-CD4 antibody (eBioscience). For the detection of apoptotic cells, Annexin V and 7-AAD were stained using a PE Annexin V Apoptosis Detection Kit I according to the manufacturer's protocol (BD Bioscience). In brief, the cells were washed with PBS and re-suspended in 100 μl annexin V-fluos labeling solution (10 mM HEPES, pH 7.4, 5 mM CaCl_2 , and 140 mM NaCl). The cells, then, were treated with 5 μl of PE-Annexin V and 5 μl 7-AAD and incubated for 15 min at RT in the dark. Stained cells were analyzed with a flow cytometer within 60 min and the data were analyzed with FlowJo version 10 software (TreeStar).

2.10. Statistical analysis

Data were presented as the mean $\pm$ s.e.m. ($n \geq 5$). The statistical analysis was performed using the ANOVA with Bonferroni's correction. The symptom score was evaluated by the Wilcoxon Rank Sum test. All data analyses were undertaken with SigmaStat software version 12 (Systat Software, Inc, Point Richmond, CA). In all comparisons, * $p < 0.05$ and ** $P < 0.01$ were used to determine statistical significance.

3. Results

3.1. PAG alleviates allergic symptoms in ovalbumin (OVA)-induced food allergy mice

When systemic anaphylaxis was induced in sensitized mice via OVA challenge, the temperature dropped rapidly within 10–30 min and allergic symptoms appeared in OVA-induced allergic mice (Fig. 1B and C). The decrease in body temperature was inhibited by PAG in a dose-dependent manner (Fig. 1B). The symptom score increased by OVA challenge was also significantly inhibited by PAG, compared to the control mice (Fig. 1C). In addition, OVA induced diarrhea in more than 80% of allergic mice, but the diarrhea was suppressed by PAG in a dose-dependent manner (Fig. 1D). These results suggested that PAG has an effect of inhibiting food allergy.

3.2. PAG inhibits the production of mast cell protease-1, histamine, and OVA-specific antibodies in OVA-induced food allergy mice

It is well known that mast cell protease-1 (MCP-1), plays an important role in histamine release from mast cells, eosinophil recruitment, and the induction of allergic response [13–15]. The amount of serum MCP-1 was largely increased in OVA-induced food allergy mice, as compared to the normal mice. However, the amount of MCP-1 was dose-dependently decreased by the administration of PAG (Fig. 2A). Histamine is a critical mediator that causes allergic symptoms in food allergy [15–17]. The concentration of serum histamine in OVA-induced food allergy mice was significantly suppressed by the administration of PAG (Fig. 2B). IgE and IgG1 antibodies are also known to be associated with inducing allergic symptoms in mice [18,19]. We next examined whether PAG inhibits the production of antibodies in OVA-induced food allergy mice. The amounts of OVA-specific IgE and IgG1 were significantly increased in OVA-induced food allergy mice, compared to the normal mice (Fig. 2B and C). The amounts of the antibodies in serum were decreased by the administration of PAG, depending on the dose of PAG administered (Fig. 2C). In addition, serum levels of IgG2a, which are classified as antibodies related to the Th1 immune response [18,19], were also increased in food allergy mice, compared to the normal mice. The serum concentration of the antibody was also decreased in the

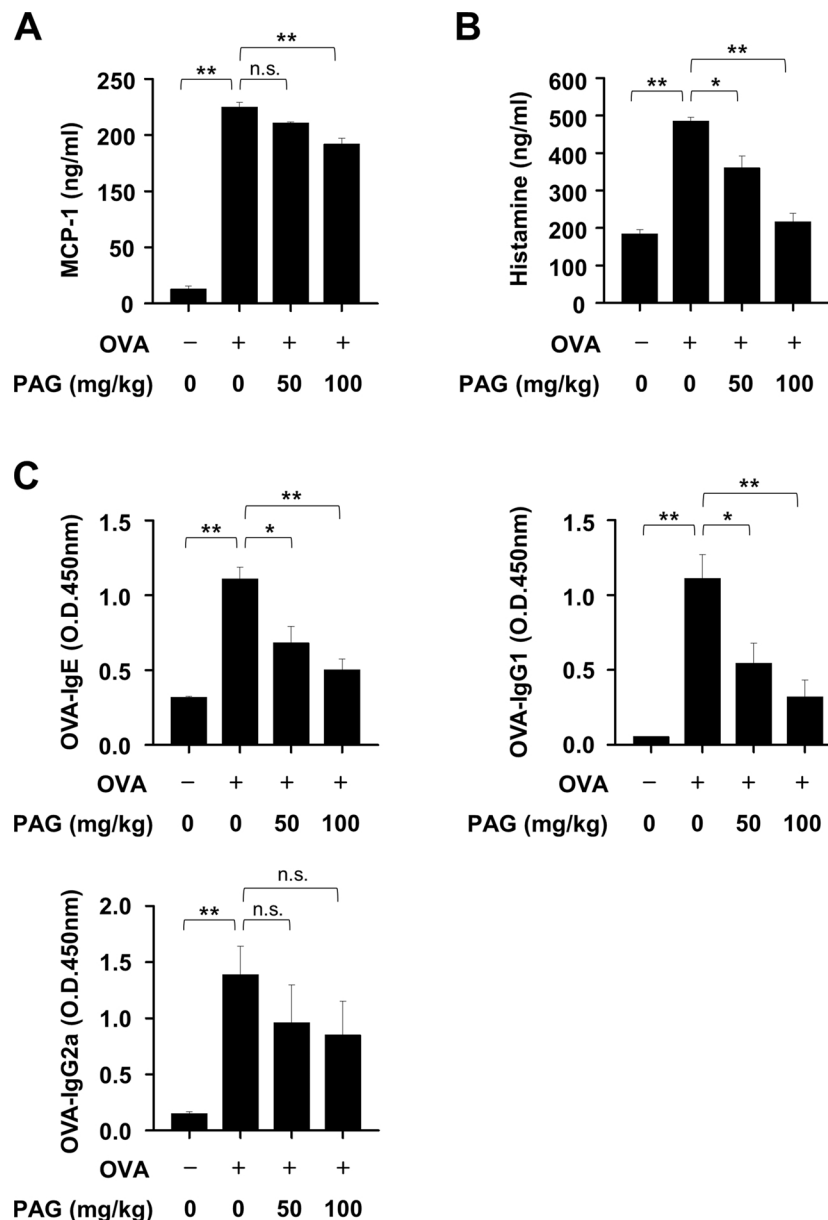


Fig. 2. PAG inhibits the secretion of allergic mediators and OVA-specific antibodies. (A–C) The amounts of mouse MCP-1 (A), histamine (B), and OVA-specific antibodies (C) were measured after the final challenge of OVA as in Fig. 1. Data were presented as the mean $\pm$ s.e.m. obtained from 3 individual experiments. $n = 5$ mice per group. * $p < 0.05$; ** $p < 0.01$; n.s., not significant. OVA, Ovalbumin; PAG, Processed *Aloe vera* gel.

group administered with PAG, but there was no statistical significance (Fig. 2C).

3.3. PAG inhibits mast cell degranulation and recruitment of eosinophil in jejunum of OVA-induced food allergy mice

It was reported that the recruitment of white blood cells to the gastrointestinal tract is increased in course of food allergy [20]. Based on the report, we measured the degranulation of mast cells and the infiltration of eosinophils in jejunum caused by food allergy and assessed the inhibitory effect of PAG using histology and flow cytometry. The number of mast cells was increased in OVA-induced food allergy mice, compared to the normal mice, but there was no significant difference (data not shown). The degranulation of mast cells in OVA-induced food allergy mice was increased, compared to the control group, and it was significantly inhibited by the administration of PAG (Fig. 3A and B). In addition, CD11b⁺ Siglec-F⁺ eosinophils were increased in OVA-induced food allergy mice compared to the normal group. This

increase was significantly inhibited at the high dose of PAG (Fig. 3C and D). These results indicated that PAG also inhibited the degranulation of mast cells and the infiltration of eosinophils in intestinal tissue caused by food allergy.

3.4. PAG suppresses the production of Th2 cytokines in OVA-induced food allergy mice

Food allergy is well known as a Th2-mediated immune response that is caused by food antigens [21,22]. The secretion of Th2 cytokines in various allergic diseases is known to play an important role in the development of allergic symptoms. IL-4, a typical Th2 cytokine, contributes to the proliferation and activation of mast cells. It is also reported that IL-4 plays an essential role in allergic reactions, such as increasing the expression of FcεRI and protein content of granules in mast cells [23]. In addition to IL-4, cytokines such as IL-5 and IL-13 secreted by Th2 cells are reported to be involved in mucus secretion, muscle contraction, and proliferation of eosinophils and mast cells as

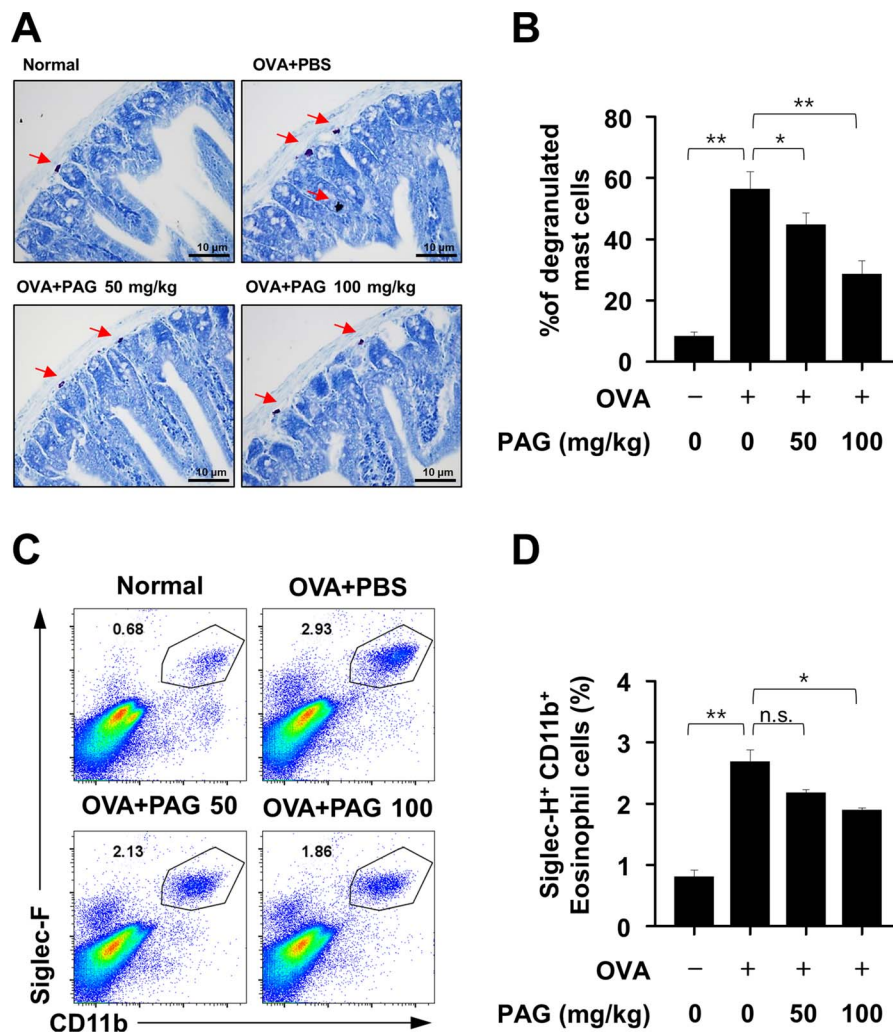


Fig. 3. PAG suppresses the accumulation of mast cells and eosinophils in jejunum. The food allergy was induced with OVA as in Fig. 1. (A, B) Representative histologic images (A) and the percentage of degranulated mast cells (B) in jejunum are shown. (C, D) Representative images from flow cytometric analysis (C) and frequency of mucosal eosinophils in jejunum after the last challenge of OVA are shown. Data are presented as the mean $\pm$ s.e.m. ($n = 5$ per group). * $p < 0.05$; ** $p < 0.01$; n.s.; not significant. OVA, Ovalbumin; PAG, Processed *Aloe vera* gel.

well as food antigen-specific IgE production [21]. Therefore, we evaluated the inhibitory effect of PAG on the secretion of Th2 cytokines in OVA-induced food allergy mice. The amount of serum IL-4 was significantly increased in OVA-induced food allergy mice compared to the normal mice (Fig. 4A). However, when the PAG was administered, the amount of IL-4 was decreased in a dose-dependent manner (Fig. 4A). In addition, the increase of IL-5 and IL-3 was also suppressed by the PAG-treated mice in a dose-dependent manner (Fig. 4B and C). On the other hand, the concentration of IFN- γ , a typical Th1 cytokine, was not changed by the induction of food allergy, and no meaningful change was observed by administration of PAG (Fig. 4D).

In addition, we measured the mRNA expression of IFN- γ and IL-4 in jejunum, which is important for decreasing body temperature and inducing diarrhea when food allergy occurs [24,25]. The expression of IL-4 mRNA was increased in jejunum of OVA-induced food allergy mice compared to the normal mice, and the expression of IL-4 mRNA was significantly inhibited by the treatment of PAG (Fig. 4E). However, the expression of IFN- γ mRNA in jejunum from normal and food allergy mice was comparable (Fig. 4F). These results suggest that PAG also inhibits Th2 immune responses in intestinal tissues from OVA-induced food allergy mice.

3.5. PAG inhibits the activation of Th2 cells isolated from OVA-induced food allergy mice

Next, we measured the effect of PAG on cytokine production in cells extracted from spleen and mesenteric lymph node in OVA-induced food allergy mice. The production of Th2 cytokines IL-4, IL-5, and IL-13 from splenocyte was increased in food allergy mice compared to the normal mice, and decreased in the mice treated with PAG (Fig. 5A). In the mesenteric lymph node cells, the amount of IL-4, IL-5, and IL-13 production was increased in the food allergy mice, as compared to the normal mice. This increase was significantly reduced in the mice treated with PAG (Fig. 5B). On the other hand, the production of IFN- γ did not show any significant difference between normal and food allergy mice with or without PAG (Fig. 5A and B).

Based on these results, we examined the effect of PAG on the distribution of Th1/Th2 cells in OVA-induced food allergy mice. The population of Th2 cells was significantly increased in splenocyte from food allergy mice. However, in mice given PAG, the population of Th2 cells was decreased to a level similar to that of the normal mice (Fig. 5C and D). In the mesenteric lymph node cells, the Th2 cell population was also significantly increased in food allergy mice. The increased Th2 cell population was significantly suppressed by PAG (Fig. 5C and D). However, the Th1 cell population from spleen and mesenteric lymph node showed no significant difference between normal mice and food

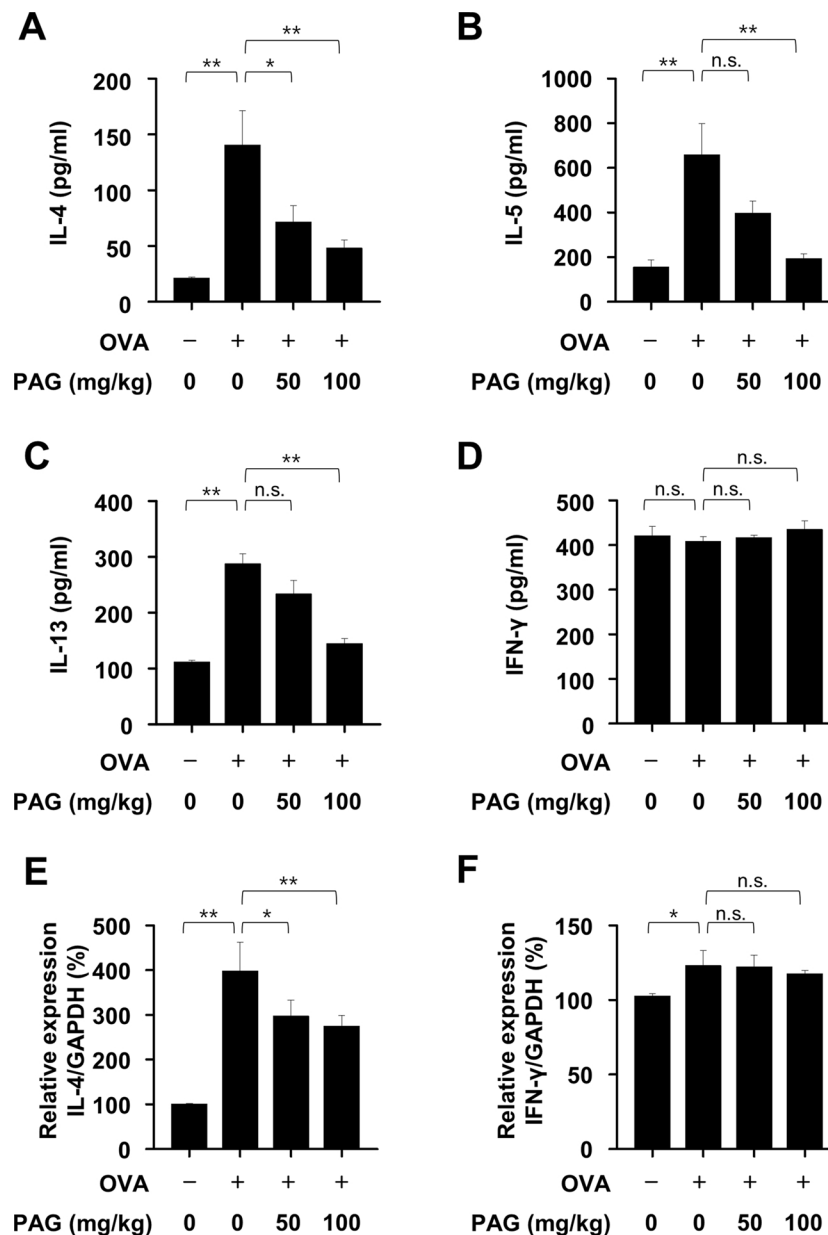


Fig. 4. PAG decreases the amounts of Th2 cytokines in OVA-induced food allergy mice. The food allergy was induced with OVA as in Fig. 1. (A–D) Amounts of IL-4 (A), IL-5 (B), IL-13 (C), and IFN-γ (D) were assessed in serum 1 h after the last challenge of OVA as in Fig. 1. (E, F) The expression of IL-4 (E) and IFN-γ (F) was measured by real time RT-PCR in jejunum. Data are presented as the mean $\pm$ s.e.m. (n = 5 per group). *p < 0.05; **p < 0.01; n.s.; not significant. OVA, Ovalbumin; PAG, Processed *Aloe vera* gel.

allergy mice (Fig. 5C and D). Activation-induced apoptosis of CD4⁺ T cells also showed no significant difference between PAG-treated and untreated cells (Fig. 5E and F). Therefore, it is suggest that PAG suppresses the increase of Th2 cells and inhibits the production of Th2 cytokines such as IL-4 and IL-5.

3.6. PAG stimulates the production of IL-10 in OVA-induced food allergy mice

Next, we investigated how PAG inhibits Th2 immune responses in food allergic mice. IL-10 is a well-known cytokine that suppresses a variety of immune responses including inhibition of Th2 immune responses [26–28]. The IL-10 production in food allergy mice was partially reduced compared to the normal mice. Surprisingly, however, the IL-10 production increased in a dose-dependent manner in mice treated with PAG in a dose-dependent manner (Fig. 6A).

IL-10 is known to be secreted in a variety of cells. In this

experiment, we examined the effect of PAG on the population of IL-10-producing Tr1 in food allergy mice. In splenocyte, IL-10-producing Tr1 cells were not altered by administration of PAG. However, in mesenteric lymph nodes, we observed a significant increase of the population of Tr1 in mice treated with PAG (Fig. 6B). Furthermore, IL-10 secreted from splenocytes and mesenteric lymph node cells was significantly increased by the administration of PAG, which is consistent with an increase in the Tr1 population (Fig. 6C). These results suggest that PAG stimulates Tr1 in food allergy mice to increase, at least partially, the production of IL-10 for the inhibition of allergic responses.

4. Discussion

Symptoms of allergic diseases such as food allergy, asthma, rhinitis, and atopic dermatitis occur in various body organs. Allergy is generally considered a disease caused by impaired Th1/Th2 immune response balance. The Th2 immune environment is a condition of the body that is

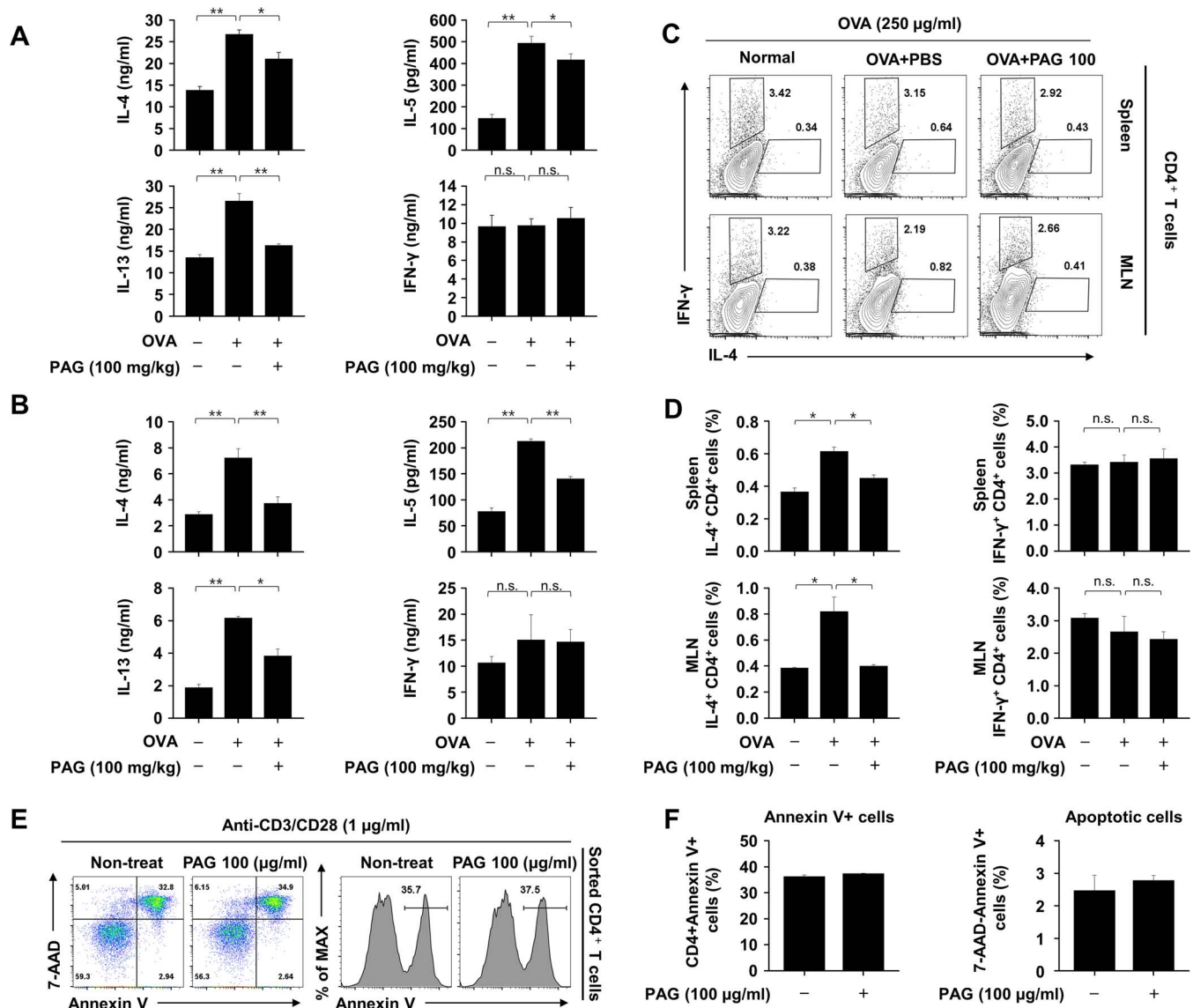


Fig. 5. PAG inhibits OVA-specific Th2 immune response in lymphoid tissues. (A–B) Cells isolated from spleen (A) and MLN (B) after the last challenge of OVA were stimulated for 72 h with or without 250 μ g/ml OVA. IL-4, IL-5, IL-13, and IFN- γ were measured in culture media by ELISA. (C–D) Representative images (C) and frequency (D) of IFN- γ ⁺ Th1 and IL-4⁺ Th2 cells by flow cytometric analysis are shown. (E–F) Representative images (E) and frequency (F) of apoptotic CD4⁺ T cells with or without PAG treatment by flow cytometric analysis are shown. Data are presented as the mean $\pm$ s.e.m. (n = 5 per group). *p < 0.05; **p < 0.01; n.s.; not significant. OVA, Ovalbumin; PAG, Processed *Aloe vera* gel.

prone to allergic diseases [18,29]. Aloe polysaccharide, a component of aloe, is reported to have therapeutic effects in a variety of immune disorders [30]. The polysaccharide stimulates immature DC maturation and activation processes. It is also associated with direct activation of B cells and NK cells [31]. Aloe polysaccharides have the effect of enhancing the immune response by activation of antigen presenting cells such as dendritic cells or macrophages [32]. In addition, aloe polysaccharide is reported to maintain the intestinal immune system in a healthy state, to strengthen the intestinal mucosa, and to reduce inflammation or polyps in the intestine [11].

The PAG used in this study is characterized by a significantly smaller molecular size of polysaccharides than those from native *Aloe vera* gel [11]. In general, materials with small molecular weights are better suited for use as medicines than those with high molecular weight because of their better bioavailability [33]. Recently, it has been reported that PAG exhibits excellent therapeutic or prophylactic effects in various diseases [33–35]. For example, PAG inhibited a carcinogenesis of colon by inhibiting chronic inflammation and cell cycle progression [33]. PAG also suppresses lipogenesis and inflammatory

responses [34]. Notably, PAG was effective in the treatment of diabetes by increasing the sensitivity to insulin [10,35]. However, the effect of PAG on food allergy remains to be determined.

Various protocols have been reported to produce animal models with food allergy [37–39]. The protocols that lead to most allergic animal models are similar, but the difference is that they use different types of adjuvant to sensitize mice. Torii et al. sensitized mice using LPS with the antigen to induce food allergy [37]. However, the most commonly used adjuvants are cholera toxin [38] and Alum [39,40]. It has been reported that cholera toxin is used as an adjuvant when sensitized by oral administration and Alum is used as an adjuvant when sensitized by intraperitoneal injection [39,40]. Besides, Alum is known to stimulate the Th2 immune response in vivo [41,42]. In this study, therefore, we used Alum as the adjuvant to sensitize in ovalbumin-induced food allergy mice (Fig. 1A).

The onset of intestine-associated allergy by OVA is facilitated by mediators including cytokines secreted by Th2 cells, mast cells, and various inflammatory cells [36,43]. Activated Th2 cells, which are increased in the body when food allergy occurs, secrete Th2 cytokines,

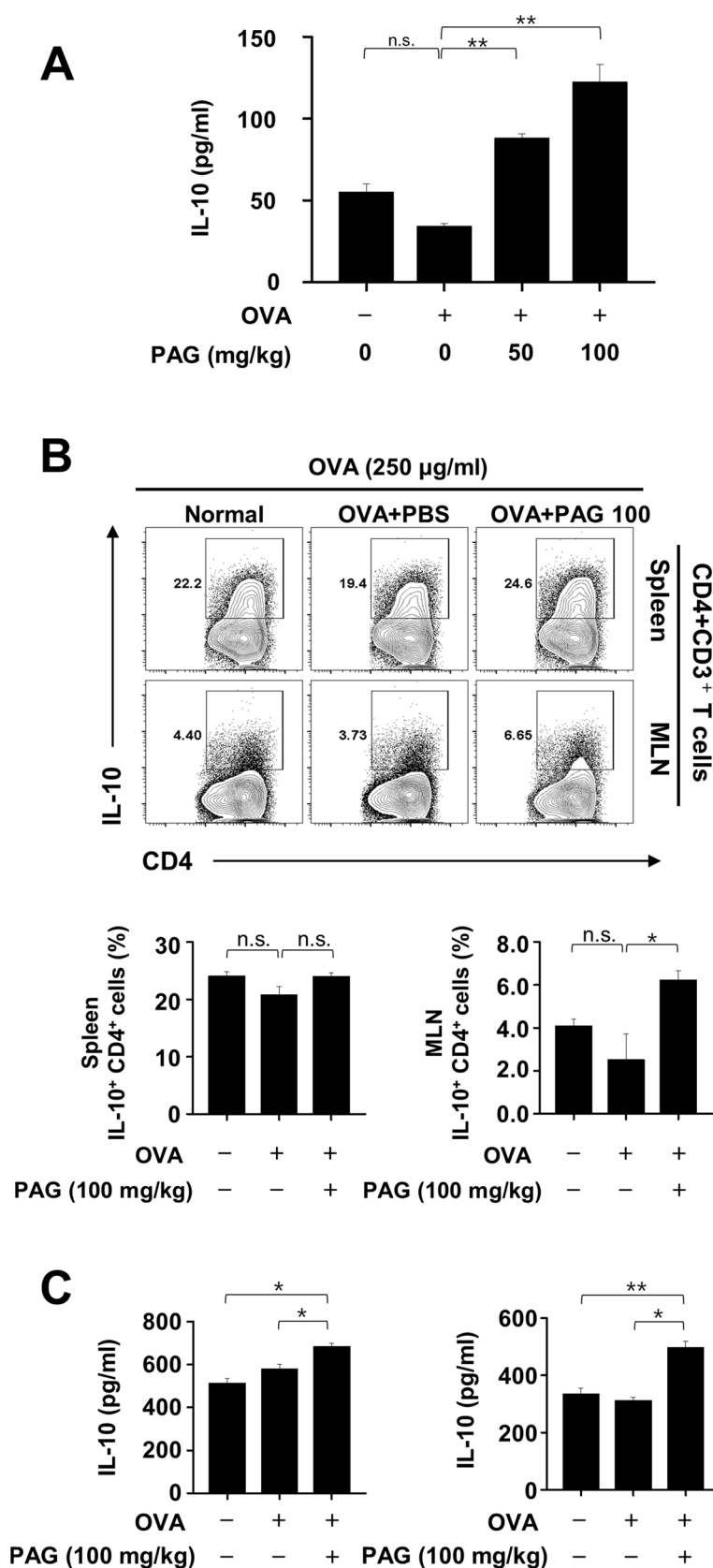


Fig. 6. PAG enhances the activation of IL-10-producing Tr1 cells. The food allergy was induced with OVA as in Fig. 1. (A) The amount of IL-10 was measured in serum 1 h after the last challenge of OVA. (B) Flow cytometric analysis of IL-10-producing CD4⁺ T cells by the challenge of OVA in the spleen and mesenteric lymph nodes. Representative FACS images (upper) and frequency of cells (lower) are shown. (C) Cells isolated from spleen and MLN after the last challenge of OVA were stimulated for 72 h with or without OVA (250 µg/ml). The amount of IL-10 was measured in culture media by ELISA. Data are presented as the mean $\pm$ s.e.m. (n = 5 per group). *p < 0.05; **p < 0.01; n.s.; not significant. OVA, Ovalbumin; PAG, Processed *Aloe vera* gel.

which cause gastrointestinal inflammation leading to bowel dysfunction [33,44]. IL-4 and IL-13 secreted from Th2 cells also increase the sensitivity of blood vessels to mediators that induce allergic reactions, thereby increasing allergic symptoms. Thus, when allergic reactions are

induced in IL-4-primed animals, allergic symptoms are largely increased [33]. We observed almost complete inhibition of allergic diarrhea when we administered PAG to food allergy mice (Fig. 1D). We also found that the administration of PAG significantly reduced the

symptoms of respiratory depression and swelling around eyes and mouth with itching (Fig. 1C). In serum, PAG significantly inhibited the amounts of OVA-specific IgE and IgG1. In addition, PAG also reduced histamine and Th2 cytokines in the serum of food allergy mice (Figs. 2 and 4). These results suggest that PAG inhibits the immunologic pathway for the production of Th2 cytokines associated with the induction of allergic symptoms in mice.

Increased numbers of intraepithelial lymphocytes, mucosal mast cells, and eosinophils in food allergy have been observed in both human patients and animal models [34]. Eosinophil migration into gastrointestinal tract and activation of mast cells in food allergy play an important role in the development of allergic symptoms [34]. Our results showed that when food allergy was developed, the activation of mast cells and the recruitment of eosinophils increased in small intestine of OVA-induced food mice (Fig. 3). However, the mast cell degranulation and eosinophil accumulation in the small intestine were suppressed by the administration of PAG (Fig. 3). Notably, in vitro cell culture experiments showed that PAG had no inhibitory effect on the activation of mast cells or eosinophils (data not shown). These results suggest that the inhibitory effect of PAG on OVA-induced food allergy is not due to a direct effect on mast cells or eosinophils, but rather because of other mechanisms.

IL-10 is a representative immunosuppressive cytokine. IL-10 inhibits the action of pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α [45,46]. In addition, IL-10 is known to reduce expression of MHC class II molecules and various co-stimulatory molecules in macrophages and dendritic cells [45,46]. IL-10 also suppresses the production of IL-4 and IL-5 by inhibiting the activity of Th2 cells [47]. Besides, IL-10 suppresses B cell differentiation and antibody production [48]. Considering these reports, IL-10 is suggested to play an important role in the treatment of allergic inflammation and asthma. It was very surprising for us that IL-10 was significantly increased in serum from OVA-induced allergy mice when PAG was administered (Fig. 6A). Thus, our results suggest that increased IL-10 by PAG inhibits Th2 cytokine production to suppress OVA-induced food allergic symptoms.

A variety of cells in our body, such as Th cells, regulatory B cells, and dendritic cells can produce IL-10. Among them, IL-10-producing type 1 regulatory T cells (Tr1), an important subset of regulatory T cells, exhibit some distinct features different from CD4⁺ CD25⁺ Foxp3⁺ regulatory T cells and serve to control allergic inflammation. Tr1 is known to suppress eosinophil, mast cell, and basophil in allergic diseases through IL-10 [49,50]. In our results, IL-10 was elevated in serum in food allergy mice by PAG administration (see Fig. 6A). Kim et al. also reported that Tr1 has a function of suppressing food allergy [4], so we focused on Tr1 among various IL-10-producing cells. In food allergy mice, we measured IL-10-producing Tr1 in immune tissues and found that the population of IL-10-producing Tr1 was increased by PAG treatment (Fig. 6B). Furthermore, we found that IL-10 secretion increased when isolated CD4⁺ Th cells from immune tissues were treated by PAG in vitro (Fig. 6C). Our results suggest that PAG stimulates Tr1 in food allergy mice and increases the secretion of IL-10 (Fig. 6). The IL-10 can contribute, at least partially, to the suppression of allergic responses by OVA in mice. However, since various cells secrete IL-10 in our body, further study on the mechanism of PAG is needed in the future.

Aloe polysaccharides have long been used as a food [51,52] and there are no reports of serious side effects including allergic reactions, as far as we know. We also did not observe adverse effects, including weight changes, behavioral abnormalities, and other allergic reactions in mice during the course of our experiment (Fig. 1A). PAG has been certified as a food product by the Korean government. Taken together, PAG is considered to be a very safe material. However, further studies on side effects that we do not know yet should be continued.

In conclusion, our results suggest that the increase of IL-10 by administration of PAG is closely associated with the inhibitory effect of PAG on food allergic symptoms. Increased IL-10 by PAG inhibits Th2 cells and consequently blocks activations of B cells, mast cells, and

eosinophils, which play critical roles to induce food allergy symptoms. However, the possibility that IL-10 directly inhibits B cells, mast cells, and eosinophils cannot be ruled out. Taken together, PAG regulates food allergy by inhibiting the Th2 immune responses, most possibly, through the increase of IL-10.

Conflict of interest

The authors have declared that there is no conflict of interest.

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